The University of Chicago
FOUNDED BY JOHN D. ROCKEFELLER

SOME OBSERVATIONS ON CATALASE

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL
OF SCIENCE IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY)

BY
CHARLES O. APPLEMAN

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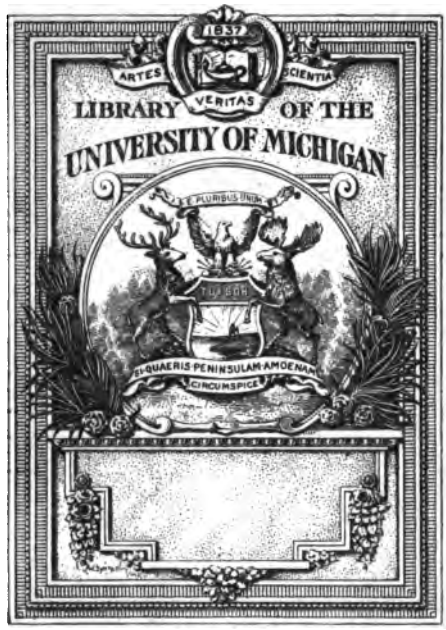

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SOME OBSERVATIONS ON CATALASE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 139

CHAS. O. APPLEMAN

(WITH ONE FIGURE)

During the course of an investigation now in progress on the physiological behavior of enzymes in after-ripening of the potato tuber, it became necessary to investigate fully the best method for the quantitative determination of catalase in this organ. Some of the results thus obtained may be of general interest.

DISTRIBUTION AND FUNCTION OF CATALASE

SCHOENBEIN (1863) was the first to observe the power of various vegetable and animal extracts to decompose hydrogen peroxid with evolution of oxygen. He concluded that the enzymes occurring in the organisms were responsible for this phenomenon. This power of hydrogen peroxid decomposition was considered a more or less general property of enzymes until LOEW (1) showed that this property of tissues is due to a special enzyme to which he gave the name catalase. Its claim for a place in the category of enzymes at this time seems to be based wholly upon its sensitiveness toward heat, acids, and various poisons.

Catalase is probably the most widely distributed of any of the known enzymes. In fact, its occurrence is so general that LOEW concluded that there did not exist a group of organisms, or any organ, or even a single vegetable or animal cell, that did not contain some catalase. An enzyme of such general occurrence might naturally be supposed to possess an important function in the economy of nature. This may yet prove to be the case, but at present its position in this respect is uncertain. Little is even known regarding its mode of action on hydrogen peroxid. Definite knowledge on this point is limited to the fact that molecular instead of atomic oxygen results from the decomposition, and in this respect it differs from the other hydrogen peroxid catalysts.

Since the decomposition of hydrogen peroxid is the most important property of this powerful enzyme so widely distributed, it was suggested by LOEW that it may possess the function of preventing the accumulation of this toxic substance in the tissues. He conceived it possible and highly probable that hydrogen peroxid was produced in the living cells as the result of respiratory processes. USHER and PRIESTLY (9) were able to demonstrate the presence of hydrogen peroxid during photosynthesis if the catalase were previously destroyed. This fact would seem to support the above theory, but on the other hand, BACH and CHODAT (2) have shown that hydrogen peroxid is not a violent poison in tissues, since they have been able to cultivate certain plants in a medium containing 0.68 per cent of it. Catalase is found also in anaerobic organisms, a further fact which rendered the above conception untenable. Other authors have ascribed to catalase the function of protection against the peroxids of the organism, thus preventing injurious oxidations. It is unable, however, to decompose the substituted organic peroxids, such as ethyl hydroperoxid or the oxygenases (2).

Probably the most important question in connection with catalase at the present time is whether or not it may be considered as an oxidizing enzyme. It is true that it does not respond to the tests with the ordinary reagents for oxidizing enzymes, but this, according to LOEW, does not militate against its being an oxidizing enzyme, since the action of oxidizing enzymes is sometimes quite specific. He claims that a characteristic oxidation by catalase is produced with hydroquinone and also some with glucose and citric acid. SHAFFER (3) thinks that this quinone-formation was undoubtedly due to the presence of some enzyme other than catalase, since he found that animal tissues always contain catalase, but frequently possess no power to oxidize hydroquinone.

According to the BUCHNER and MEISENHEIMER (4) conception of alcoholic fermentation, the sugar is converted into lactic acid by the zymase, and the lactic acid in turn is split up into alcohol and carbon dioxid by a lactacidase enzyme. In a recent work KOHL (5) claims to have proved, for yeast fermentation at least, that catalase performs the function of the zymase in the above

conception, and if no zymase is present the lactic acid is oxidized to oxalic acid by oxidizing enzymes. If zymase is present, it splits the salts of lactic acid up into alcohol and carbon dioxid, the work of the lactacidase according to BUCHNER and MEISENHEIMER. This work of KOHL'S, if confirmed, brings to catalase a very important rôle in physiological processes as an oxidizing enzyme.

METHODS

The following method was employed in all the catalase determinations except where otherwise stated. The apparatus used is shown in fig. 1. The potato was grated rapidly on a nutmeg grater with frequent dipping of the grated surface into calcium carbonate. After grating, the pulp was ground for two minutes in a mortar with quartz sand. The extract was then pressed lightly through two layers of absorbent cotton and one of cheese cloth. After mixing, 1 cc. was withdrawn immediately and placed into the bottle used for the determination, and 1 cc. of *cold* water added. The apparatus was then placed into a water bath at 20° C. After the apparatus had attained the temperature of the bath, 5 cc. of Oakland 3 per cent hydrogen peroxid (dioxygen) were run into the bottle from the separating funnel *2*. The stopcock *a* was opened 15 seconds before the minute. On the minute, shaking was begun and continued with as much regularity as possible to the end of the experiment. On the quarter-minute the stopcock *b* was opened and the oxygen allowed to run into the gas burette. On the half-minute the stopcock *b* was closed and a reading made at the burette. Stopcock *c* was now opened until the menisci in the burettes were again level. At the three-quarter mark the stopcock *b* was opened again and closed on the minute for a reading. This procedure was continued with readings every 30 seconds for three minutes. With longer periods the experimental error becomes greater on account of lack of uniform shaking.

EFFECT OF GRINDING WITH CALCIUM CARBONATE

GRÜSS (10) reports that it is impossible to make quantitative determinations of catalase in the fresh potato extract on account of the rapid degeneration during the grinding and subsequently.

If the potato is ground with calcium carbonate to neutralize the acids freed by the grinding, and 5 cc. of the extract is at once diluted with 50 cc. and kept at a temperature of 20° C. or below, this rapid degeneration may be overcome and comparable results obtained

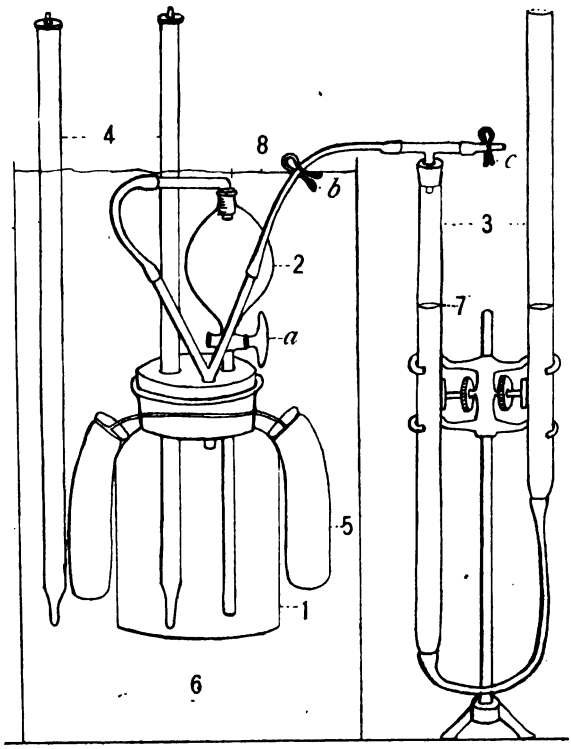

FIG. 1.—Apparatus used for catalase determinations: *1*, thin-walled bottle; *2*, separatory funnel; *3*, gas burettes; *4*, thermometers; *5*, weights; *6*, water bath; *7*, water meniscus; *8*, water level in bath; *a*, stopcock for running the hydrogen peroxid into bottle; *b*, stopcock for controlling the flow of oxygen into the burette; *c*, stopcock for equalizing the air pressure in burettes.

without any difficulty. It is necessary, however, to maintain the same period of time for grinding, as there is a slight degeneration even under the above conditions, due to another cause, as we shall see later. The shaking must also be constant and uniform during the determination.

Table II shows the effect of grinding and storage with calcium carbonate. After grinding two potatoes of equal weight, one with

and the other without calcium carbonate, they were stored at 0° C. for 34 days. At the end of this time, 200 cc. of water were

TABLE I

LOSS OF CATALASE DUE TO ACIDS IN THE EXTRACT

POTATO	CC. O_2 EVOLVED IN 1-MIN. PERIODS					TOTAL CC. O_2 IN 5 MIN.
	1st	2d	3d	4th	5th	
Ground without $CaCO_3$....	3.2	3.0	2.3	1.7	1.3	11.5
Same extract after standing 15 min.....	1.7	1.8	1.6	1.3	1.0	7.4
Ground with $CaCO_3$.....	6.4	5.9	4.6	3.6	2.8	23.3
Same extract after standing 90 min.....	6.5	5.8	4.3	3.3	2.6	22.5

added to each flask containing the pulp, and 5 cc. of this dilution and 5 cc. of hydrogen peroxid were used for the determination in each case.

TABLE II

EFFECT OF STORAGE FOR 34 DAYS AT 0° C. WITH AND WITHOUT CALCIUM CARBONATE

POTATO	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
	1st	2d	3d	4th	5th	6th	
Ground without $CaCO_3$.....	0.3	0.4	0.4	0.4	0.4	0.4	2.3
Ground with $CaCO_3$	12.4	10.9	8.9	6.9	5.4	4.0	48.4

FILTERING

Approximately 50 per cent of the total catalase will pass through an ordinary filter paper, but none through a Chamberland-Pasteur filter. The first fact seems to indicate the existence of an insol-

TABLE III

EFFECT OF FILTERING THROUGH ORDINARY FILTER PAPER

EXTRACT OF POTATO TUBER	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
	1st	2d	3d	4th	5th	6th	
Filtered through filter paper	2.8	2.4	1.8	1.5	1.3	1.1	10.9
Portion of same extract unfiltered.....	4.0	4.5	3.8	3.2	3.1	3.4	22.6

uble (α-catalase) and a soluble (β-catalase) form. KOHL finds the same true of yeast catalase, and LOEW believes it a general char-

acter of vegetable catalase. The size of the molecule of the latter prevents its passage through a Chamberland-Pasteur filter.

Table IV also shows the effect of filtering and the necessity of thorough mixing of the extract before taking the sample for a total catalase determination.

TABLE IV

EFFECT OF FILTERING AND SETTLING

EXTRACT OF POTATO TUBER	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
	1st	2d	3d	4th	5th	6th	
Unfiltered	12.2	9.5	7.0	5.0	4.0	3.2	40.9
Supernatant liquid after settling	5.4	4.5	3.5	3.0	...	...	....
After mixing, 20 min. later	11.9	9.5	6.9	5.3	3.8	3.2	40.6
Filtered, 40 min. later	5.5	4.8	3.5	2.7	2.1	1.7	10.3

TABLE V

EFFECT OF FILTERING THROUGH A CHAMBERLAND-PASTEUR FILTER

EXTRACT OF POTATO TUBER	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
	1st	2d	3d	4th	5th	6th	
a) Unfiltered	24.8	22.8	19.3	16.8	14.8	13.5	52.
b) Filtered through Chamberland filter	0.	0.	0.	0.	....	....	....
c) Residue of (*b*) made up to same volume as (*a*)	25.	22.3	19.3	16.7	14.8	13.5	51.6

TEMPERATURE RELATIONS

Catalases from different sources show considerable variation in temperature relations, the point of total destruction in the cases reported ranging from 65° C. to 80° C. In potato catalase, however, destruction is complete when the temperature reaches 50° C.

The VAN'T HOFF velocity coefficient for hemase has been found to be 1.5. The same figure applies to potato catalase, but is evident only from 0 to 10°. At 20° a destruction of the catalase begins, which renders the accelerating effect of higher temperatures upon the peroxid decomposition impossible of manifestation.

The diminishing coefficient indicated in table VI is due to actual destruction of the catalase, as will be seen by table VII, which also shows that the destruction at moderate temperatures is not due

to impurities in the hydrogen peroxid. Some substance in the potato may be freed by the grinding and brought into contact

TABLE VI

JOINT EFFECT OF ACCELERATION AND DESTRUCTION BY RISING TEMPERATURE

Range of temperature	0–10°	10–20°	20–30°	30–40°
Velocity coefficients	1.5	1.1	0.85	0.18

with the catalase, which effects its slow destruction. It is interesting to note in this connection, however, that SENTER (6) found hemase to be oxidized at all temperatures above 0° C.

TABLE VII

AMOUNT OF CATALASE DESTRUCTION AT 30° C. FOR 15 MIN. OVER THAT AT 20° C. FOR THE SAME PERIOD

POTATO TUBER EXTRACT	DETERMINATION TEMP.	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
		1st	2d	3d	4th	5th	6th	
a) Exposed to 30° C. only during determination of catalase	30° C.	14.8	14.7	14.1	13.2	12.5	11.9	21.2
b) Exposed to 30° C. during exp. (*a*), then cooled to 20° C. for determination	20° C.	15.0	14.5	13.7	13.3	12.7	12.4	21.6
c) Not exposed above 20° C	20° C.	15.9	15.1	14.7	13.9	13.3	12.8	25.7

DESTRUCTION OF THE CATALASE DURING THE REACTION WITH HYDROGEN PEROXID

Potato catalase is not unlimited in its power to effect the decomposition of hydrogen peroxid. Table VIII shows not only the above fact, but also would seem to indicate that it is consumed in the reaction and that a given amount is capable of decomposing a definite amount of hydrogen peroxid.

The total extract of a potato weighing 50 grams was diluted to 500 cc. with water, and 5 cc. of this extract were allowed to act upon 1 cc. of 1.5 per cent hydrogen peroxid until less than 0.1 cc. of oxygen was evolved in 5 min. Another cubic centimeter of hydrogen peroxid was then added and the reaction allowed to run to the same point. This procedure was continued until the addition of a cubic centimeter of hydrogen peroxid produced only 1.1 cc. of oxygen in 25 min.; 6 cc. were required to bring the reaction to

this point. A fresh portion of 5 cc. of the extract, which had stood at the same temperature, was now allowed to act upon 6 cc. of hydrogen peroxid at the same time, with the result that approximately the same amount of oxygen was evolved as the total produced when 6 cc. were added in successive lots of 1 cc. each. The experiment was repeated several times with different extracts and dilutions, with practically the same result in every case.

TABLE VIII

CONSUMPTION OF THE CATALASE DURING THE REACTION WITH HYDROGEN PEROXID

POTATO TUBER EXTRACT	CC. O_2 EVOLVED WHEN H_2O_2 WAS ADDED IN SUCCESSIVE LOTS OF 1 CC. EACH						TOTAL CC. O_2 EVOLVED	TOTAL CC. O_2 EVOLVED WHEN 6 CC. WERE ADDED IN BULK
	1st	2d	3d	4th	5th	6th		
5 cc. extract	8.2	6.5	6.3	5.4	4.0	1.1	31.6	
5 cc. same extract	...	...	...	...	...	...	....	31.8

The destruction or degeneration of the catalase would be slight during the time of the experiment in the above dilute neutral solution at 20°. Admitting a slight destruction, it would be the same in both cases, and would therefore not affect the general result. KASTLE (7) suggests that catalases, like peroxidases, may combine with the hydrogen peroxid to form an unstable holoxid derivative, as $K+H_2O_2=H_2KO_2$. This might in turn oxidize the catalase itself (K) which may be more readily oxidizable than any of the peroxidase reagents, in which event we would have $H_2KO_2=H_2O+KO$. This conception is in striking harmony with the facts indicated in the above table, and with the fact that the reaction is decidedly an exothermal one.

RELATION TO FUNCTIONAL ACTIVITY

There is considerable evidence from animal tissues that catalase activity bears a relation to functional activity of the structure. Such a relation seems to exist in the potato, at least in respect to respiratory activity. Extracts from potatoes which have been kept for several days at 0° C. show a decided decrease in catalase activity. MÜLLER-THURGAU (8) has also shown that respiration is greatly reduced in such potatoes.